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DEGRADATION AND IMPURITY PROFILE REPORT:
HEPES

N02 PROCESS VALIDATION: BSI-PRL-0400

DEGRADATION AND IMPURITY PROTOCOL:
BSI-PRL-0436

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1. PURPOSE AND SCOPE:

- 1.1. The impurity profiling of HEPES was intended to identify and possibly quantify impurities found in the HEPES product manufactured and purified at the BioSpectra facility.
 - 1.1.1. In the case where an impurity was found, a limit was set to the maximum allowable for establishing as pure of a product as possible. In the case where a limit could not be set, a procedure was written and followed, to identify if the possible impurity was present or not (i.e. an identity test, which is qualitative.).
 - 1.1.2. The profiling results and data allowed BioSpectra to further understand the purity and characteristics of HEPES.
 - 1.1.3. The four stages of HEPES that were tested are Raw Material, Mother Liquor, Wet Crystals and the Finished Goods.
 - 1.1.4. The tests and samples analyzed to determine the presence of impurities and degradation products were as follows:
 - 1.1.4.1. Absorbance (0.1M)
 - 1.1.4.1.1. RM (each lot), Mother Liquor, WC 1st basket, FG Beginning Drum
 - 1.1.4.2. Appearance and Color
 - 1.1.4.2.1. Raw Material (each lot) and FG Beginning Drum
 - 1.1.4.3. Assay/pKa
 - 1.1.4.3.1. Raw Material (each lot), Mother Liquor, and FG Beginning Drum
 - 1.1.4.4. Elemental Impurities (USP<232>) with Addition of Calcium, Iron, Magnesium, Manganese, Potassium, and Zinc.
 - 1.1.4.4.1. RM (each lot), Mother Liquor, WC 1st Basket, FG Beginning Drum
 - 1.1.4.5. Identification (HPLC)
 - 1.1.4.5.1. Raw Material (each lot) and FG Beginning Drum
 - 1.1.4.6. Identification (IR)
 - 1.1.4.6.1. RM (each lot), Mother Liquor, WC 1st Basket, FG Beginning Drum
 - 1.1.4.7. Insoluble Matter
 - 1.1.4.7.1. Raw Material (each lot) and FG Beginning Drum
 - 1.1.4.8. Loss on Drying
 - 1.1.4.8.1. Raw Material (each lot) and FG Beginning Drum
 - 1.1.4.9. pH of a 5% Solution
 - 1.1.4.9.1. Raw Material (each lot) and FG Beginning Drum
 - 1.1.4.10. Residual Solvents: Methanol and Ethanol
 - 1.1.4.10.1. Raw Material (each lot) and FG Beginning Drum
- 1.2. All results were recorded in the appropriate laboratory documentation. The results were detailed and analyzed in this report. This report includes all relevant data as well as references to the initial documented results. This report discusses any impurities found in the product and includes a specification for any limits on the impurities found when applicable.

2. RESPONSIBILITIES:

- 2.1. The Executive Director of Quality Control was responsible for control, implementation, and maintenance of this procedure. The Executive Director of Quality Control was responsible for ensuring the completion of the degradation and impurity testing report.
- 2.2. The QC Analysts were responsible for performing the testing stated in the protocol and recording all results in the appropriate laboratory documentation.

3. REFERENCES:

- 3.1. BSI-ATM-0054, Analytical Method of Analysis: Hepes via ICP-MS
- 3.2. BSI-ATM-0070, HEPES Testing Methods
- 3.3. BSI-FRM-0849, HEPE-3200 HEPES Validation Summary Sheet
- 3.4. BSI-PRL-0400, HEPES Process Validation Protocol Bangor 2021-N02
- 3.5. BSI-PRL-0436, Degradation and Impurity Profile Protocol: HEPES N02 Process Validation
- 3.6. BSI-RPT-0845, Elemental Impurity Assessment HEPES N02 2021
- 3.7. BTOI21-176, Manufacturing TOI for Batch

4. PROCEDURE:**4.1. ABSORBANCE (0.1M):**

- 4.1.1. Refer to the Degradation and Impurity Profile Protocol: HEPES for testing methods and requirements. The results of the Absorbance 0.1M testing are detailed in the table below.

TABLE 1: ABSORBANCE (0.1M)

Lot Number	Stage of Material	Specification	Result		
			250 nm	260 nm	280 nm
RMAT-1021-0028	Raw Material	Monitor	0.0040	<0.003	<0.003
RMAT-1021-0030			0.0012	<0.003	<0.003
RMAT-1021-0033			0.0080	0.0035	0.0023
HEPE-0121-00154-PV ML	Mother Liquor	Monitor	0.0613	0.0241	0.0172
HEPE-0121-00154-PV WC Basket 1	Wet Crystal	Monitor	0.0010	<0.003	<0.003
HEPE-0121-00154-PV Re-dried Drum 1	Finished Good	0.0500 a.u. max @ 250nm and 260 nm 0.0800 a.u. max @ 280nm	0.0069	0.0023	0.0016

4.2. APPEARANCE AND COLOR:

- 4.2.1. Refer to the Degradation and Impurity Profile Protocol: HEPES for testing methods and requirements. The results of the Appearance and Color testing are detailed in the table below.

TABLE 2: APPEARANCE AND COLOR

Lot Number	Stage of Material	Specification	Result
RMAT-1021-0028	Raw Material	Monitor	White/Crystals
RMAT-1021-0030			White/Crystals
RMAT-1021-0033			White/Crystals
HEPE-0121-00154-PV Re-dried Drum 1	Finished Good	White/Crystals	White/Crystals

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4.3. ASSAY (Dried)/pKa:

4.3.1. Refer to the Degradation and Impurity Profile Protocol: HEPES for testing methods and requirements. The results of the Assay/pKa testing are detailed in the table below.

TABLE 3: ASSAY (DRIED)/PKA

Lot Number	Stage of Material	Specification		Result	
		Assay	pKa	Assay	pKa
RMAT-1021-0028	Raw Material	Monitor		100.49%	7.500
RMAT-1021-0030				100.42%	7.499
RMAT-1021-0033				100.58%	7.501
HEPE-0121-00154-PV ML	Mother Liquor	Monitor		54.91%	7.50
HEPE-0121-00154-PV Re-dried Drum 1	Finished Good	99.5% minimum	7.45- 7.65	100.2%	7.51

4.4. ELEMENTAL IMPURITIES

4.4.1. Refer to the Degradation and Impurity Profile Protocol: HEPES for testing methods and requirements. The results of the Elemental Impurities are detailed in the table below.

TABLE 4: ELEMENTAL IMPURITIES

Lot Number	Stage of Material	Specification	Result
RMAT-1021-0028	Raw Material	Report for Ca, Fe, Mg, Mn, K, Zn	Refer to BSI-RPT-0845 for Elemental Impurity Assessment
RMAT-1021-0030			
RMAT-1021-0033			
HEPE-0121-00154-PV ML	Mother Liquor		
HEPE-0121-00154-PV WC Basket 1	Wet Crystal		
HEPE-0121-00154-PV Re-dried Drum 1	Finished Good		

4.5. IDENTIFICATION (HPLC):

4.5.1. Refer to the Degradation and Impurity Profile Protocol: HEPES for testing methods and requirements. The results of the Identification (HPLC) testing are detailed in the table below.

TABLE 5: IDENTIFICATION (HPLC)

Lot Number	Stage of Material	Specification	Result
RMAT-1021-0028	Raw Material	Monitor	Conforms
RMAT-1021-0030			Conforms
RMAT-1021-0033			Conforms
HEPE-0121-00154-PV Re-dried Drum 1	Finished Good	Passes Test	Passes Test

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4.6. IDENTIFICATION (IR):

4.6.1. Refer to the Degradation and Impurity Profile Protocol: HEPES for testing methods and requirements. The results of the Identification (IR) testing are detailed in the table below.

TABLE 6: IDENTIFICATION (IR)

Lot Number	Stage of Material	Specification	Result
RMAT-1021-0028	Raw Material	Monitor	Passes Test; 0.998313
RMAT-1021-0030			Passes Test; 0.998355
RMAT-1021-0033			Passes Test; 0.996423
HEPE-0121-00154-PV ML	Mother Liquor	Monitor	0.915257
HEPE-0121-00154-PV WC Basket 1	Wet Crystal	Monitor	0.995204
HEPE-0121-00154-PV Re-dried Drum 1	Finished Good	Passes Test	Passes Test; 0.999511

4.7. INSOLUBLE MATTER:

4.7.1. Refer to the Degradation and Impurity Profile Protocol: HEPES for testing methods and requirements. The results of the Insoluble Matter testing are detailed in the table below.

TABLE 7: INSOLUBLE MATTER

Lot Number	Stage of Material	Specification	Result
RMAT-1021-0028	Raw Material	Monitor	0.0035%
RMAT-1021-0030			<0.0010%
RMAT-1021-0033			<0.0010%
HEPE-0121-00154-PV Re-dried Drum 1	Finished Good	0.01% maximum	0.0020%

4.8. LOSS ON DRYING:

4.8.1. Refer to the Degradation and Impurity Profile Protocol: HEPES for testing methods and requirements. The results of the Loss on Drying testing are detailed in the table below.

TABLE 8: LOSS ON DRYING

Lot Number	Stage of Material	Specification	Result
RMAT-1021-0028	Raw Material	Monitor	<0.0158%
RMAT-1021-0030			0.0407%
RMAT-1021-0033			0.1141%
HEPE-0121-00154-PV Re-dried Drum 1	Finished Good	0.5% maximum	0.0249%

4.9. pH (5% SOLUTION):

4.9.1. Refer to the Degradation and Impurity Profile Protocol: HEPES for testing methods and requirements. The results of the pH (5% Solution) are detailed in the table below.

TABLE 9: PH (5% SOLUTION)

Lot Number	Stage of Material	Specification	Result
RMAT-1021-0028	Raw Material	Monitor	5.28 @ 24.0°C
RMAT-1021-0030			5.27 @ 24.3°C
RMAT-1021-0033			5.25 @ 23.9°C
HEPE-0121-00154-PV Re-dried Drum 1	Finished Good	5.0-6.5	5.3 @ 23.2°C

4.10. RESIDUAL SOLVENTS**USP<467><1467>:**

4.10.1. Refer to the Degradation and Impurity Profile Protocol: HEPES for testing methods and requirements. The results of the Residual Solvent testing are detailed in the table below.

TABLE 10: RESIDUAL SOLVENTS

Lot Number	Stage of Material	Specification Meets USP<467><1467>	Result	
			Methanol	Ethanol
RMAT-1021-0028	Raw Material	Methanol Ethanol	<1510 ppm	None Detected
RMAT-1021-0030	Raw Material		<1510 ppm	None Detected
RMAT-1021-0033	Raw Material		<1510 ppm	None Detected
HEPE-0121-00154-PV Re-dried Drum 1	Finished Good		None Detected	None Detected
Meets USP<467><1467>				

5. CONCLUSION:

- 5.1. All samples met the specifications for the required analyses as dictated in the Degradation and Impurity Profile Protocol: HEPES.
- 5.2. It can be concluded that there are no additional identifiable impurities present in the HEPES material at any stage of the process at this time.
- 5.3. HEPE-0121-00154-PV batch was Re-dried in accordance with BTOI21-176. The Degradation and Impurity Testing was performed on the Re-dried Finished Good Material.